

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(19) Japan Patent Office (JP)
 (12) **Unexamined Japanese
 Patent Application (A)**

(11) Japanese Patent Application
 Kokai Publication No.
Sho 60-220068

(51) Int. Cl.⁵
 A 61-L 15/01

ID Symbol

JPO file No.
 6779-4C

(43) Date of publication: November 2, 1985

Examination Request: Not filed. No. of Inventions: 3 (Total of 9 pages)

(54) Title of the invention: **COATING FILM FOR LIVING TISSUES**

(21) Application No.: Sho 59-75073

(22) Filing Date: April 16, 1984

(72) Inventor:	IWATA, Mitsuo ⁱ	16-1-1218 Minami-kasai 3-chome, Edogawa-ku, Tokyo
(72) Inventor:	TAKAHASHI, Akira ⁱⁱ	5-28 Tsujidou-shinmachi 3-chome, Fujisawa-shi
(72) Inventor:	UMEMOTO, Akiko ⁱⁱⁱ	14-6 Choufugaoka 2-chome, Choufu-shi
(72) Inventor:	AOGI ^{iv} , Juurou ^v	16-45 Narita-nishi 1-chome, Suginami- ku, Tokyo
(71) Applicant:	TERUMO CORPORATION	44-1 Hatagaya 2-chome, Shibuya-ku, Tokyo
(74) Agent:	Patent Attorney NISHIMURA, Kouyuu ^{vi}	

SPECIFICATION

1. Title Of The Invention

Coating film for living tissues

2. Claims

- (1) A coating film for living tissues consisting of a graft copolymer film made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500.
- (2) The coating film for living tissues according to claim 1, in which the above-mentioned water-soluble polymer is a polymer selected from polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, and alginic acid.
- (3) The coating film for living tissues according to claim 1, in which the above-mentioned graft copolymer film is a film having a thickness of 5 to 1,000 μm , a moisture permeability of 0.1 to 200 $\text{mg}/\text{cm}^2\cdot\text{hr}$, and a water absorption of 0.1 to 150 g/cm^2 .
- (4) A coating film for living tissues consisting of:
 a graft copolymer film layer made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500, and
 a biocompatible support film layer formed on top of said graft copolymer film layer.
- (5) The coating film for living tissues according to claim 4, in which the above-mentioned water-soluble polymer is a polymer selected from the group comprising polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, and alginic acid.

(6) The coating film for living tissues according to claim 4, in which the above-mentioned support film layer is made of a polymer selected from nylon, polypropylene, polyethylene, polyester, urethane, SBS, polyvinyl chloride, cellulose, acrylic, natural or synthetic rubber, and thermoplastic elastomers.

(7) A coating film for living tissues consisting of:

a graft copolymer film layer made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500,

a biocompatible support film layer formed on top of said graft copolymer film layer, and

a moisture permeation control layer formed on top of said support film layer or between said support film layer and the above-mentioned graft copolymer film layer.

(8) The coating film for living tissues according to claim 7, in which the above-mentioned water-soluble polymer is a polymer selected from the group comprising polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, and alginic acid.

(9) The coating film for living tissues according to claim 7, in which the above-mentioned support film layer is made of a polymer selected from nylon, polypropylene, polyethylene, polyester, urethane, SBS, polyvinyl chloride, cellulose, acrylic, natural or synthetic rubber, and thermoplastic elastomers.

(10) The coating film for living tissues according to claim 7, in which the above-mentioned moisture permeation control layer is formed from natural rubber, synthetic rubber, or thermoplastic elastomer.

(11) The coating film for living tissues according to claim 10, in which the above-mentioned moisture permeation control layer is formed from silicone rubber, urethane rubber, SBS, or EPDM.

3. Detailed Explanation Of The Invention

I. Background Of The Invention

Technological Field

The present invention relates to a novel coating film used for living tissues. More specifically, the present invention relates to a coating film for living tissues consisting of a graft copolymer film made up of a water-soluble polymer and a solubilized keratin.

When the skin is damaged by wounds, burns, etc., normally, an ideal solution is to treat it by taking the person's own skin from other sites on the body and transplanting it. However, there are limitations on the quantity of the skin and the sites, from where it can be taken, which is why artificial coating films are commonly used when the injured area is large in size. The coating film of the present invention is used for the protection and treatment of such damaged skin.

Prior art

In the past, lyophilized pig skin, nylon sheets, silicone gauze, silicone rubber film, film made from coagulated blood serum, fibrin film, oil-treated gauze, etc. have been used as coating films for living tissues for the above-described purposes. However, they all had various problems in terms of their compatibility with injured areas, water vapor permeability, ability to prevent bacterial infections, etc. In addition, coating films utilizing collagen have been recently proposed (U.S. Pat. No. 4280954). Coating films made of collagen possess superior properties from the standpoint of biocompatibility. However, collagen exhibits antigenicity and is insoluble in water. Atherocollagen is a collagen treated to eliminate its antigenicity, but it has the disadvantage that the preparation^{vi} of the film in this is quite difficult. Namely, atherocollagen does not permit preparation of high-

concentration aqueous solutions and if the pH is not near 3, it does not dissolve in water, and thus requires neutralization procedures later. Additionally, due to its high viscosity, it is difficult to handle, and when it is rendered insoluble, it is not easy to control crosslinking reactions occurring in it. In addition, it is expensive and its adhesion to the skin is not very good.

II. Object Of The Invention

Thus, it is an object of the present invention to provide an inexpensive and easy-to-manufacture coating film for living tissues superior in its water-vapor permeability (moisture permeability), compatibility with and adhesion to the skin, ability to prevent bacterial infections, etc. Furthermore, it is an object of the present invention to provide a coating film for living tissues that is superior in its ability to be absorbed by living tissue and exhibits no antigenicity. The coating film for living tissues of the present invention, which attains the above-described objects, consists of a graft copolymer film made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500. Furthermore, the coating film for living tissues of the present invention consists of the above-mentioned graft copolymer film and a biocompatible support film layer formed on top of it. In addition, the coating film for living tissues of the present invention consists of the above-mentioned a graft copolymer film layer, a biocompatible support film layer formed on top of it, and a moisture permeation control layer formed on top of said support film layer or between said support film layer and the above-mentioned graft copolymer film layer.

Furthermore, in the coating film for living tissues of the present invention, the above-mentioned graft copolymer film has a thickness of 5 to 1,000 μm , a moisture permeability of 0.1 to 200 $\text{mg}/\text{cm}^2\text{-hr}$, and a water absorption of 0.1 to 150 g/cm^2 . Additionally, in the coating film for living tissues of the present invention, the above-mentioned water-soluble polymer is a polymer selected from polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, and alginic acid.

Furthermore, in the coating film for living tissues of the present invention, the above-mentioned support film layer is made of a polymer selected from nylon, polypropylene, polyethylene, polyester, urethane, SBS, polyvinyl chloride, cellulose, acrylic, natural or synthetic rubber, and thermoplastic elastomers.

Moreover, in the coating film for living tissues of the present invention, the above-mentioned moisture permeation control layer is formed from silicone rubber, urethane rubber, SBS, or EPDM.

III. Detailed Description Of The Invention

First of all, the coating film for living tissues of the present invention is characterized by consisting of a graft copolymer film made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500.

The above-mentioned solubilized keratin is itself prepared in accordance with publicly known methods, for instance, the reduction method developed by O'Donnell *et al.* (I. J. O'Donnell *et al.* Aust. J. Biol. Sci., Vol. 17, p. 973, 1964). Namely, the keratin is obtained by adding wool to a solution of urea, treating the mixture with mercaptoethanol and then iodoacetic acid, filtering, and then subjecting the solution to dialysis and centrifugal separation. Otherwise, it can be solubilized by the oxidation method, in which wool is treated with performic acid (S. Moore, Journal of Biologi[cal] Chemistry, Vol. 238, p. 235, 1963).

Polyethylene glycol, polypropylene glycol, polyvinyl pyrrolidone, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, and alginic acid are suggested as suitable examples of the above-described water-soluble polymers. The polymers need to have a molecular weight of not less than 500, with a molecular weight of 2,000~10,000 being preferable. Polymers obtained by the

graft polymerization of a water-soluble polymer with a molecular weight of less than 500 onto keratin retain their antigenicity.

The graft copolymer of the present invention is itself prepared by publicly known methods. For instance, when the water-soluble polymer is represented by polyethylene glycol, polypropylene glycol, polyacrylamide, polyvinyl alcohol, water-soluble cellulose derivatives, alginic acid, etc., the graft copolymers are obtained by reacting the above-mentioned water-soluble polymers with a coupling agent, such as, for example, cyanuric chloride, and their reaction product is then coupled with the solubilized keratin. Also, when the water-soluble polymer is represented by polyvinyl alcohol, alginic acid, water-soluble cellulose derivatives, etc., said water-soluble polymer can be subjected to graft copolymerization onto the solubilized keratin under irradiation with ionizing radiation. Furthermore, the above-mentioned graft copolymer can be obtained by reacting acrylamide, acrylic acid, vinyl pyrrolidone, and other monomers with the solubilized keratin.

Because the graft copolymer made up of the thus obtained water-soluble polymer and solubilized keratin is soluble in water, the coating film is made by crosslinking it to render it insoluble in water. Graft copolymers insoluble in water can be produced by the various methods shown below.

(1) A water-soluble graft copolymer is dissolved in water or a mixture of water and alcohol, the solution is placed in a dish and dried, and the resulting film is rendered insoluble by immersing it in an aldehyde solution, such as a glutaraldehyde solution or a formaldehyde solution, with the glutaraldehyde solution being especially preferable.

A water-soluble graft copolymer can be dissolved in water to a concentration of about 10%, but it is preferable to dissolve it to a concentration of 5%, place it in a dish and allow it to dry. The resulting film is immersed in a 25% glutaraldehyde solution for not less than 2 hours and then washed with water and dried. Drying can be carried out either by air drying or by freeze-drying.

As far as water is concerned, for convenience, distilled water can be used, but when one wants to prepare a graft copolymer solution of a higher concentration, its solubility can be increased by adjusting the pH to make it more acidic or more basic. For instance, in case of carboxymethyl-keratin graft copolymer, its solubility becomes extremely high at a pH of 1 to 2 or at a pH of 8 to 9.

(2) An aldehyde solution, in particular, a glutaraldehyde solution, is added to a water-soluble graft copolymer solution, with the mixed solution placed in dishes and allowed to dry. It is preferable to add glutaraldehyde to produce a concentration of about 0.5%.

(3) A water-soluble graft copolymer solution is placed in a dish and allowed to dry, with the resultant film slowly stretched in a water vapor atmosphere so as to make it 1.5 to 5 times (preferably, 2.5 to 4 times) larger and maintained in this state for not less than 30 minutes, preferably not less than 3 hours.

(4) A water-soluble graft copolymer solution is placed in dishes and allowed to dry, with the resulting film irradiated with γ -rays at not less than 4 Mrad (preferably, at 6 to 10 Mrad) under a de-oxygenized atmosphere or with UV rays under a nitrogen atmosphere.

(5) A water-soluble graft copolymer solution is dissolved in a carboxylic acid, in particular, in formic acid, trihaloacetic acids (for example, trichloroacetic acid, tribromoacetic acid), or dihaloacetic acids (for example, dichloroacetic acid, dibromoacetic acid) in the proportion of about 5%, and the solution is placed in dishes and dried. Although other carboxylic acids can be used, the above-mentioned carboxylic acids are highly soluble, and therefore especially preferable.

(6) The solubilized keratin obtained by severing the crosslinked portions in keratin molecules is rendered insoluble by crosslinking it again. Namely, wool is reduced with tri-*n*-butylphosphine, after which formic acid is added to the wool, which is then subjected to ultrasonic treatment. After

centrifugal separation, a coating film is formed from the supernatant. Otherwise, it can be obtained in accordance with the O'Donnell method, by reducing wool, setting the pH of the soluble portion to 5, subjecting it to dialysis and making a film by casting the solution.

(7) An insoluble polymer is obtained by dissolving a water-soluble graft copolymer in water or a mixture of water and alcohol, drying the solution, and treating the resultant film with hot water at 45°C or more.

(8) An insoluble polymer is obtained by dissolving a water-soluble graft copolymer in water or a mixture of water and alcohol, and the solution is subjected to dehydration by heating. It is desirable to carry out the thermal dehydration treatment at 45°C or more.

The degree of insolubility of the graft copolymer film and its ability to be absorbed by living tissues can be regulated by varying the degree of crosslinking in the course of the above-mentioned insolubilization treatment.

The coating film made from the thus obtained graft copolymer preferably has a thickness of 5 to 1,000 μm , a moisture permeability of 0.1 to 200 $\text{mg}/\text{cm}^2\cdot\text{hr}$, and a water absorption of 0.1 to 150 g/cm^2 .

The above-mentioned thickness of the graft copolymer film is necessary to maintain a stable strength and coating effects. Moisture permeability is necessary to prevent tissue necrosis on the surface of the wound and is represented by the amount of water vapor evaporated per unit time per unit surface area. Water absorption is necessary to absorb the excess exudate and is expressed as the amount absorbed per unit surface area of the film.

The numerical values of the above-mentioned physical properties suitable for the graft copolymer film are not necessarily critical, but they need to be within the above-mentioned numerical value ranges in order for the coating film to perform its functions properly, and are appropriately selected within the ranges depending on the situation, in which the film is used. For instance, bodily fluids exude copiously in the initial stage of a burn, moisture and heat are evaporated and dissipated by using a graft copolymer film with high water absorption and moisture permeability values.

The above-described graft copolymer film can be used as the coating film as is, however, when the situation demands that a wound remain protected for an extended period of time, it is preferable to reinforce the above-mentioned graft copolymer film by forming a biocompatible support film layer on top of the film. The biocompatible support film layer should not contain materials exhibiting toxicity or causing inflammation in the living tissue and is a layer supporting the coating film, which is gellified by absorbing bodily fluids when used for an extended period of time. Suitable materials for such a support film layer include nylon, polypropylene, polyethylene, polyester, urethane, SBS, polyvinyl chloride, cellulose, acrylic, various rubbers, and thermoplastic elastomers. The film preferably is shaped so as to permit penetration by living tissue, for example, it could be mesh, nonwoven fabric, woven fabric, velour sponge film, etc.

In order to form the above-mentioned support film layer on top of the graft copolymer, the support film is immersed in an aqueous solution of the graft copolymer, dried, and then crosslinked with glutaraldehyde; otherwise, glutaraldehyde can be added to the aqueous solution of the graft copolymer, and the support film can be then crosslinked by immersing it in the solution and drying. When the support film is difficult to wet, it can be rendered hydrophilic by plasma treatment. Furthermore, in the present invention, a moisture permeation control layer is preferably formed on top of the above-mentioned biocompatible support film layer or between said support film layer and the above-mentioned graft copolymer film layer. Preferred materials used for the above-mentioned moisture permeation control layer include silicone rubber, urethane rubber, SBS, EPDM, etc. The

moisture permeation control layer is formed such that the moisture permeability of the entire coating film is 0.1 to 100 mg/cm²·hr.

Manufacturing examples of the coating film of the present invention are described below.

Manufacturing Examples

1. Preparation of solubilized keratin

(Example 1)

95 ml of 8M urea solution with a pH of 7.4 adjusted using hydrochloric acid were added to 1.7g of wool (Wool Top) and then 0.02M tris(hydroxymethyl)aminoethane and 0.001M disodium ethylenediamine tetraacetate (EDTA-2 Na) were added to the mixture. After nitrogen substitution, 1 ml of mercaptoethanol was added thereto and the pH was adjusted to 10.3 with 5N KOH. After stirring the mixture for 3~4 hours, 2.68g of iodoacetic acid was added to it and the pH was adjusted to 8.5 with 5N KOH. After stirring the mixture overnight, the mixture was filtered through a Nutsche filter, and approximately 100~110 ml of the filtrate were subjected to dialysis over a period of 5 days. The material remaining after dialysis was subjected to centrifugal separation for 1 hour at 10,000 rpm and the supernatant was lyophilized, yielding 0.68g of solubilized keratin (yield: 40~60%).

(Example 2)

3 ml of hydrogen peroxide solution were added dropwise to 27 ml of formic acid under cooling, with the mixture subsequently subjected to agitation for 2 hours at room temperature. 1.0g of wool was immersed in the resulting formic acid solution. After allowing the mixture to stand for 24 hours under light-shielded conditions, it was filtered using a glass filter (G₃). The residue was added to 150 ml of pH11 ammonia solution and the mixture was subjected to agitation for 2 hours. The pH of the mixture was adjusted to 10.3 with ammonia, and, after stirring it for 24 hours, it was subjected to centrifugal separation for 1 hour at 10,000 rpm, whereupon the supernatant was lyophilized, yielding a solubilized keratin (yield: 30~50%).

2. Preparation^{viii} of graft copolymer

Preparation^{ix} of polyethylene glycol-keratin graft copolymer (called PEG-SCMK below)

1) Synthesis of cyanogenated polyethylene glycol

36.7g (0.2 mol) of cyanuric chloride were dissolved in 800 ml of anhydrous benzene. 20g of anhydrous sodium carbonate and 100g of polyethylene glycol (PEG from Union Carbide, molecular weight: 5,000) were added to the solution and the mixture was subjected to agitation at 30°C for 40 minutes. Upon termination of the reaction, the anhydrous sodium carbonate was filtered off and reprecipitated with petroleum ether. The precipitate was removed and dissolved in 500 ml of benzene, and then again precipitated by adding petroleum ether. This procedure was repeated until liquid chromatography could not detect cyanuric chloride any more, and the reaction product was dried in vacuo.

2) Synthesis of PEG-SCMK

3.0g of carboxymethylated solubilized keratin extracted from wool were dissolved in 300 ml of 0.1M borax (whose pH had been set to 9.6 with 6N KOH), and the solution was cooled to 5°C. 10g of the cyanogenated polyethylene glycol were slowly added to the solution over a period of 30 minutes, and the mixture was stirred for 6 hours at 5°C. The reaction solution was subjected to dialysis overnight, and the produced hydrogen chloride was removed. Potassium dihydrogenphosphate (pH 7.1) was used as the external dialysate and Bisking tubing 30/32 (manufactured by Bisking) was used as the dialysis membrane. Subsequently, the reaction solution was salted out by adding ammonium sulfate to a concentration of 11g/100 ml. The precipitate was separated by centrifugal separation (8,500 rpm, 1.5 hours) and then subjected to dialysis overnight. Water was used as the external dialysate and the above-described tubing was used as the dialysis

membrane. After confirming that unreacted polyethylene glycol had been removed, the internal dialysate was lyophilized, yielding the intended product (PEG-SCMK). The yield was 49.8%. A cytotoxicity test conducted on a 0.5% solution of the product was negative.

3. Preparation^x of coating film for living tissues

(1) Coating film for living tissues consisting of a graft copolymer film

Method A

The PEG-SCMK obtained above is dissolved in distilled water, yielding a 5% aqueous solution. The solution, in the amount of 0.16 ml/cm^2 , is placed in a Teflon dish and air dried, producing a PEG-SCMK film with an average thickness of $50\sim 60 \mu\text{m}$.

The thus obtained film is immersed in 25% glutaraldehyde for 4 hours. After thoroughly washing it with water, the target coating film for living tissues is obtained.

Method B

A glutaraldehyde solution is added to a 5% PEG-SCMK solution to produce a concentration of approximately 0.5%, whereupon the resulting solution, in the amount of 0.16 ml/cm^2 , is placed in a Teflon dish and air-dried.

Method C

Under a water vapor atmosphere, a PEG-SCMK film obtained in the same manner as in Method A is slowly stretched to make it four times larger in size and maintained in this state for 3 hours.

Method D

A PEG-SCMK film, obtained in the same manner as in Method A, is irradiated with γ -rays at 6 Mrad. In addition, a 4-W UV lamp is used to irradiate it for 3 hours with UV radiation on one side, from a distance of 10 cm, under a nitrogen atmosphere.

Method E

PEG-SCMK is dissolved in formic acid, yielding a 5% formic acid solution. Said solution, in the amount of 0.16 ml/cm^2 , is placed in a Teflon dish and air-dried.

Method F

Wool is reduced in accordance with the O'Donnell method, the pH of the soluble portion is adjusted to 5, and it is subjected dialysis, whereupon a film is fabricated by casting.

Method G

PEG-SCMK is dissolved in distilled water and the aqueous solution is placed in a dish and air-dried. The resulting film is placed in hot water at 80°C for 15 minutes, removed, and dried.

Method H

PEG-SCMK is dissolved in distilled water. The solution is placed in a dish and dehydrated/dried at a temperature of 80°C .

The physical properties of the coating films obtained by the above-described Methods A through H are listed in Table 1.

Table 1
Physical Properties Of Coating Films

Method	Thickness (μm)	Moisture Permeability ($\text{mg}/\text{cm}^2\cdot\text{hr}$)	Water Absorption (g/cm^2)
Method A	50~60	35~45	15~20
Method B	50~60	35~45	15~20
Method C	15~30	25~35	10~15
Method D	50~60	35~45	90~100
Method E	40~50	20~30	3~4
Method F	60~80	25~35	5~10
Method G	15~30	25~35	10~15
Method H	50~60	25~35	10~15

Methods of Determination

Moisture Permeability

Tests were conducted in accordance with the cup method (JIS Z1504). However, a sponge with a thickness permitting precise contact with the film was inserted in the apparatus such that water was in constant contact with the film, whereupon distilled water was poured inside. The test was conducted at a temperature of 37°C and a humidity of 45%.

Water Absorption

The films that had been allowed to stand for 24 hours in distilled water were taken out and moisture was removed from their surfaces, whereupon they were allowed to stand at a temperature of 37°C and a humidity of 45% until a stable volume was reached. The difference in the weight before the test and after the test was divided by the surface area.

(2) Coating film for living tissues comprising a graft copolymer film layer and a biocompatible support film layer

PEG-SCMK was dissolved in distilled water, yielding a 5% aqueous solution. The solution, in the amount of 0.16 ml/cm², was placed in Teflon dishes. Nylon mesh was cut into Teflon dish-sized pieces, accurately placed on top of the solution, and the samples were air-dried. The resultant films were immersed in a 25% glutaraldehyde solution for 4 hours. The samples were then washed with water for 10 minutes.

(3) Coating film for living tissues comprising a graft copolymer film layer, a biocompatible support film layer, and a moisture permeation control layer

The graft copolymer film was fabricated in accordance with the same procedure as in (2) described above. After washing with water for 10 minutes, the samples were dried (air-dried).

Any material can be used as the moisture permeation control layer so long as the material is a silicone rubber film (dimethylpolysiloxane). In this case, the material was a thin film, YE3085, manufactured by Toshiba Silicone. Its thickness (which can vary between 10 and 300 μm) was 100 μ .

A nylon mesh (NBC No. 330) was used as the support film layer. The support film layer can be bonded to the graft copolymer film in advance; in addition, it may be first pre-bonded to the silicone rubber layer. In this case, the latter method is used. The graft copolymer film is coated with a thin layer of silicone sealant (Dow 891), whereupon a silicone rubber film with a nylon mesh is immediately firmly applied and adhered thereto and then left stand under a load (overnight), resulting in a composite film of a practically sufficient bond strength.

IV. Operation And Effects Of The Invention

The coating film for living tissues of the present invention consists of a graft copolymer film made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500. It is superior in its compatibility with and ability to bond to the skin without triggering a rejection reaction in the living tissue. Depending on the method of its preparation^{xi}, the graft copolymer film used in the present invention can be assimilated and absorbed by the living tissues; on the other hand, it is superior in adhesion to wounds, leaving no cracks or gaps through which bacteria can penetrate. In addition, when it is absorbed by living tissue, there is no need to peel it off, and even if it is peeled off, it is easy to peel due to its softened state. Furthermore, it can be peeled off without disturbing the wound because it does not penetrate inside the granulation tissue of the wound.

Furthermore, although the coating film for living tissues of the present invention is based on protein as its raw material, it has the advantage of possessing no antigenicity, and, therefore, can be repeatedly applied to wound surfaces.

Moreover, in the coating film for living tissues of the present invention, the graft copolymer film has a thickness of 5 to 1,000 μm , a moisture permeability of 0.1 to 200 $\text{mg}/\text{cm}^2\cdot\text{hr}$, and a water absorption of 0.1 to 150 g/cm^2 . The above-mentioned physical properties are selected within the ranges indicated in the present invention in accordance with the intended use and depending on the condition, location, etc. of the wound. For instance, the abundant production of bodily fluids in the initial stage of a burn suggests that a coating film with high water absorption and moisture permeability values should be selected. In addition, when the wound dries up, a film with high water retention properties is selected. The film can promote therapeutic effects if it is impregnated with a therapeutic agent in solution form. In such a case, tissue necrosis can be prevented by imparting an appropriate degree of moisture permeability to the film.

In addition, because the coating film for living tissues of the present invention does not permit penetration by bacteria, it helps maintain wounds in an aseptic condition, which is extremely useful from a therapeutic standpoint.

Furthermore, the coating film for living tissues of the present invention consists of a graft copolymer film layer made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500 and a biocompatible support film layer formed on top of said graft copolymer film layer; therefore, the physical strength of the film is reinforced.

Furthermore, the coating film for living tissues of the present invention consists of a graft copolymer film layer made up of solubilized keratin and a water-soluble polymer with a molecular weight of not less than 500, a biocompatible support film layer formed on top of said graft copolymer film layer, and a moisture permeation control layer formed on top of said support film layer or between said support film layer and the above-mentioned graft copolymer film layer; therefore, the permeability of the film can be appropriately adjusted depending on the wound condition, duration of use, etc.

Experimental examples are shown next to specifically explain the above-described operation and effects of the present invention.

1) Antigenicity Test

The antigenicity of the graft copolymer of the present invention was tested using the Schultz-Dale technique. Namely, 2 ml of a 0.5W/V% test solution were administered to a guinea pig (male, 200~250g) three times at 24 hour intervals to sensitize them. One month later, approximately 7 cm of the guinea pig's ileum were harvested and placed in a Ringer-Locke solution, cleaning the inside of the ileum with a syringe. 3 cm of the ileum were cut off, tied with surgical suture, and placed in a Ringer bath, applying a slight pulling force to both ends thereof. One side was attached to a recorder

so as to permit contraction recording. Samples were added to the Ringer bath to produce a concentration of 1×10^{-5} g/ml, and the contractions of the ileum were recorded.

If ileum contractions were present, they were believed to indicate an antigen-antibody reaction (+), and when there were no contractions, it was believed that there was no antigen-antibody reaction (-). The results are listed in Table 2.

Table 2

Antigen Antibody-containing samples	A	B	C	D	E	F	G	H
A	+	-	-		-	-		+
B	-	-						
C	-		-					
D				-				
E	+				-			
F	-					-		
G							-	
H	+							+

- A: Solubilized keratin.
 B: Polyethylene glycol (Mn=2,000)-keratin graft copolymer
 C: Polyethylene glycol (Mn=5,000)-keratin graft copolymer
 D: Polyvinyl pyrrolidone (Mn=40,000)-keratin graft copolymer
 E: Polyvinyl pyrrolidone (Mn=9,000)-keratin graft copolymer
 F: Polyhydroxyethyl methacrylate (5,000)-keratin graft copolymer
 G: Polyacrylamide (Mn=3,000)-keratin graft copolymer
 H: Polyethylene glycol (Mn=300)-keratin graft copolymer.

As can be seen from Table 2, solubilized keratin (A) and polyethylene glycol (Mn=300)-keratin graft copolymer (H) exhibit antigenicity, while the rest of the water-soluble polymer-keratin graft copolymers do not.

2) Biocompatibility Test

Subcutaneous skin pockets were made on the backs of female guinea pigs (about 200g). 1 cm \times 1 cm samples were implanted therein, and the wound was closed.

Upon lapse of a certain period of time, the skin of the guinea pigs was cut open and peeled off to examine the condition of the samples. The results are listed in Table 3.

Table 3

Coating Film Samples		(1) Degree of absorption	(2) Rejection Reaction	(3) Granulation tissue formation
Graft Copolymers	Insolubilization Method			
B	Crosslinking with glutaraldehyde after preparation of film	3	-	2
C	Preparation of film after crosslinking with glutaraldehyde	2	-	2
E	Crosslinking by irradiation with γ -rays	3	-	2
G	Preparation of film after crosslinking with glutaraldehyde	4	-	2
Control (lyophilized pig skin)		3	-	4

- (1) Absorption
 1. No changes in the sample
 2. No changes in shape despite a certain decrease in strength.
 3. 1/3~1/2 was absorbed, or completely softened.
 4. Some of the sample remained.
 5. None of the sample remained.
- (2) Rejection reaction
 - Absolutely no reaction.
 - ± Slight rejection reaction.
 - + Acute rejection reaction.
- (3) Granulation tissue formation
 1. None at all.
 2. Slight granulation tissue formation.
 3. Granulation tissue formed over the entire surface of the sample.
 4. A thick layer of granulation tissue formed over the entire surface of the sample.

As can be seen from Table 3, during subcutaneous implantation, the coating film for living tissues of the present invention, in the same manner as the control, exhibited no rejection reactions whatsoever. The absorption of the samples in the living tissue varied depending on the method of fabrication, but was either identical or higher than that of the control. In addition, the term "absorption" used here refers to a phenomenon, whereby the film loses its ability to act as a coating film when implanted inside living tissues or bonded to a wound etc. for several weeks or longer. For instance, its strength becomes extremely low, and it partially "melts away." The formation of granulation tissue is one of the reactions of the body to the implantation of foreign material. Such tissue is formed in large amounts upon implantation and disappears as the foreign material is assimilated and absorbed. If there is some granulation tissue remaining, scars are formed, which is why it is preferable that such tissue should disappear as quickly as possible. Table 2^{xiii} shows that the coating film of the present invention is superior in this respect. In addition, the same results were obtained for films with a thickness of 5 to 1,000 μm , a moisture permeability of 0.1 to 200 $\text{mg}/\text{cm}^2\cdot\text{hr}$, and a water absorption of 0.1 to 150 g/cm^2 .

3) Bacterial permeability test

Agar-agar	15.0g
Sodium chloride	5.0g
Papain digestion product of soybean powder	5.0g
Pancreatin digestion product of casein	15.0g

The coating film samples shown in Table 3 were placed on top of TSA cultures made up of the above-described components and 1 ml of a $10^7/\text{ml}$ *Serratia marcescens* (*Serratia marcescens*) suspension was injected in the dishes. Three hours later, the bacterial solution was removed from each of the film samples, and the culture was grown at 31°C.

When the growth of the bacteria was examined 24 hours later, no bacterial growth was found in any of the cases, in which the films were used.

4) Adhesion Test

A 2 × 2 cm strip of skin was removed from the back of a rat. A sample sized to completely cover it was placed on the spot, covered with gauze, and affixed to the body with tape. 72 hours later, the strength of adhesion of the sample was measured. The method of measurement consisted in pulling one edge of the sample in the vertical direction at a constant speed (20 cm/min) while measuring the stress generated at such time.

The results are listed in Table 4.

Table 4

Coating Film Samples			Stress (g)
Graft Copolymer	Insolubilization Method	Support Film Layer	
C	Preparation of film after crosslinking with glutaraldehyde	Nylon mesh	142
E	Crosslinking with glutaraldehyde after preparation of film	"	133
None		"	117
B		Polyester nonwoven fabric	139
None	Crosslinking with glutaraldehyde after preparation of film	"	125

As can be seen from Table 4, the coating film for living tissues of the present invention consisting of a graft copolymer film layer and a biocompatible support film layer formed on top of it possesses proper skin adhesion and is superior for use as an artificial coating film.

Patent Applicant
Agent

TERUMO CORPORATION
Patent Attorney Kouyuu NISHIMURA

Translator's Notes

- ⁱ Also pronounced "Teruo." – trans.
- ⁱⁱ Also pronounced as "Aki," "Atsushi," "Kou," "Kouji," "Mitsuru," "Noboru," "Teru," etc. – trans.
- ⁱⁱⁱ Other pronunciations are "Mitsuko," "Shouko," and "Teruko." – trans.
- ^{iv} Other pronunciations include "Aoyag," and "Aeyagi." – trans.
- ^v Other pronunciations are "Shigerou," "Shigeo," and "Shigeaki." – trans.
- ^{vi} Other pronunciations are "Masao" and "Yukio." – trans.
- ^{vii} Literally, "adjustment." In Japanese, the words "preparation (fabrication)" and "adjustment" are pronounced identically, hence the typographical error. – trans.
- ^{viii} See Note VII. – trans.
- ^{ix} See Note VII. – trans.
- ^x See Note VII. – trans.
- ^{xi} See Note VII. – trans.
- ^{xii} Sic; must be "Table 3." – trans.